Synergistic Interaction of Trimethoprim and Sulfamethoxazole on *Paracoccidioides brasiliensis*

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The in vitro interaction of trimethoprim and sulfamethoxazole on clinical isolates of *Paracoccidioides brasiliensis* was studied. With complete inhibition and a visual endpoint used as the criteria, three of four strains had minimal inhibitory concentrations that indicated resistance to sulfamethoxazole, and all four strains were resistant to trimethoprim. A marked synergism in inhibition was noted with the combination of these drugs against sulfa-resistant strains. A sulfamethoxazole/trimethoprim ratio of 5:1 was the most synergistic. Fifty percent inhibition, determined spectrophotometrically, of the strains could be achieved with sulfamethoxazole alone. In summary, the striking synergy observed suggests that combination chemotherapy with these drugs deserves further study.

*Paracoccidioidomycosis* (South American blastomycosis) is the most widespread systemic mycosis in Latin America (4). Imported cases are reported in the United States (8, 15). The fungal phase in vivo is a yeast. The disease appears in two forms, as an acute or chronic pulmonary disease or disseminated, with involvement of the mucocutaneous tissues, lymphoreticular system, and other organs (19). It is believed that the active disease is progressive and, without treatment, ultimately fatal (15, 17). The initial therapeutic agents used to treat it were sulfa drugs. However, even with a criterion of partial inhibition, in vitro resistance of the etiological agent (*Paracoccidioides brasiliensis*) to achievable serum concentrations is not uncommon (16), and efficacy could not be demonstrated in an animal model (24). Although clinical experience has been obtained largely with the sulfa drugs, these agents are now thought to be suppressive at best in most cases because of slow clinical responses, failures to respond, and relapses after apparently successful therapy (20, 23). Amphotericin B and miconazole produce high response rates, but they must be given intravenously and have undesirable side effects (25), and relapse is also a problem (23, 26). Recent reports of success with ketoconazole are encouraging (21); however, although ketoconazole can, like the sulfa drugs, be given orally, it is more expensive.

Because a synergistic interaction is so commonly demonstrated between sulfa drugs and trimethoprim, side effects with the combination are few, and oral therapy is possible (22), the present study of the in vitro interaction of these agents with *P. brasiliensis* was undertaken.

The strains used were clinical isolates from Colombian patients (21, 26). Minimum inhibitory concentrations (MICs), determined by a visual endpoint, and the 50% inhibitory concentration (IC₅₀), determined by a spectrophotometric method, were assayed as previously described (10) by using the yeast phase in an inoculum of 10⁶ cells per ml at an incubation temperature of 35°C. Modified liquid McVeigh-Morton medium, a synthetic medium lacking antagonists to sulfa action (18), was used to grow the inoculum and in the assays. Trimethoprim and sulfamethoxazole in commercially available combination tablets provide peak serum levels in the ratio of 1:20, respectively (22). As this ratio may not provide optimal synergistic interaction for some organisms, e.g., *Nocardia* (3), ratios of 1:5 and 1:2 were also studied. Twofold dilutions from a starting concentration of 1,600 or 2,000 μg of each drug per ml were used in different runs. Each in vitro determination was performed two to four times, and in no case did the result vary by more than two dilutions from the highest to the lowest value in all studies. The mean value of the multiple assays is given in each case, and if that value fell between dilutions, the arithmetic mean is given. Incubation for 5 to 7 days was required before growth was clearly evident in the control (drug-free) tubes.

Table 1 shows the results of MIC determinations with sulfamethoxazole alone, trimethoprim alone, and the combination in various propor-
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2:1 5:1 20:1

3.9-0.78 31.3-1.6

4.6-9.4 23.4-1.2

15.6-3.1 500-25

0.97-0.20 0.97-0.05

S, Sulfamethoxazole; T, trimethoprim.

With regard to the first of these findings, only isolate 263 came from a patient previously treated with sulfa. This patient relapsed after apparent response earlier to sulfa. The third finding discussed above was most easily understood by calculation of a fractional inhibitory concentration (FIC) index, in a fashion similar to that described previously (6), and this allowed comparison with the data obtained previously with these drugs on bacteria (5) (Table 2). The FIC for each drug equals the MIC of the drug alone divided into its concentration in a particular combination at the lowest concentrations of that combination inhibiting growth, and the index equals the sum of the two MICs. By this formulation, an index of 1.0 represents an additive effect, >2.0 shows antagonism, and <1.0 shows synergy. Examination of Table 2 thus readily reveals the marked synergy for three strains.

When the IC₅₀ was determined, resistance to trimethoprim alone was still evident (Table 3) for three strains, but resistance to sulfamethoxazole alone would not have been as evident by this parameter. This is of interest because of the convention in in vitro sulfa susceptibility testing of using 80% inhibition of bacteria as a cutoff, with the implication that there may be a large discrepancy between the concentrations required for partial inhibition and complete inhibition (27). Presumably in relation to our finding of inhibition with sulfamethoxazole, the IC₅₀ of the drugs in combination was no more striking, with inhibition demonstrated at dilutions of approximately 1.0 µg/ml or less of both drugs or, for

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<th>TABLE 2. FIC indices for sulfamethoxazole and trimethoprim</th>
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* S, Sulfamethoxazole; T, trimethoprim. As the MIC for S was off-scale (>2,000 µg/ml) for three isolates, an arbitrary value of 4,000 µg/ml was used for these FIC calculations, shown by a < symbol.

In summary, a variety of factors were important in the clinical setting of the present work. Several findings were apparent. (i) Three of four and four of four isolates were resistant to achievable serum concentrations of sulfamethoxazole and trimethoprim, respectively. (ii) Marked synergy was noted with the three strains resistant to sulfamethoxazole at all ratios tested. (iii) In two instances, the 5:1 ratio gave the most impressive results; in a third (strain 263), the 20:1 ratio was best, but only slightly better than 5:1 was; in the other, susceptibility to sulfamethoxazole dominated the results, and the isolate appeared indifferent to the effect of the small amounts of trimethoprim at that end of the dilution series. (iv) Each isolate was susceptible to at least one combination at concentrations achievable in serum with higher doses of the drugs (11), doses that have been utilized in other human diseases (1, 7, 12).

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<th>TABLE 3. IC₅₀ for sulfamethoxazole and trimethoprim alone and in combination</th>
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* S, Sulfamethoxazole; T, trimethoprim.
strain 263, at the IC₅₀ of the more potent of the two drugs (sulfamethoxazole).

Although it is not clear without further study which in vitro parameter (MIC or IC₅₀) is most relevant for in vivo effect or what inhibitory drug concentration will correlate with in vivo outcome, the results suggested the following: (i) Combination therapy potentially offers advantages over sulfa therapy alone; (ii) a more extensive survey of strains should utilize the 5:1, and possibly also the 20:1, sulfamethoxazole/trimethoprim ratio to substantiate our results; (iii) even for those strains which are susceptible to sulfa, the combination, although not improving the sulfa effect, does not detract from it; and (iv) unlike the experience with bacteria (22), the efficacy of the combination does not appear to be dependent on the level of trimethoprim susceptibility. Therapeutic studies in an animal model could resolve whether the powerful synergistic interaction demonstrated here will translate into an in vivo effect greater than that with sulfa drugs alone. Although several reports (2, 9, 13, 14) of good results in human paracoccidioidomycosis with this drug combination have appeared, it cannot yet be said with certainty, in the absence of randomization, that these results are superior to what would have been attained with sulfa drugs alone.

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LITERATURE CITED